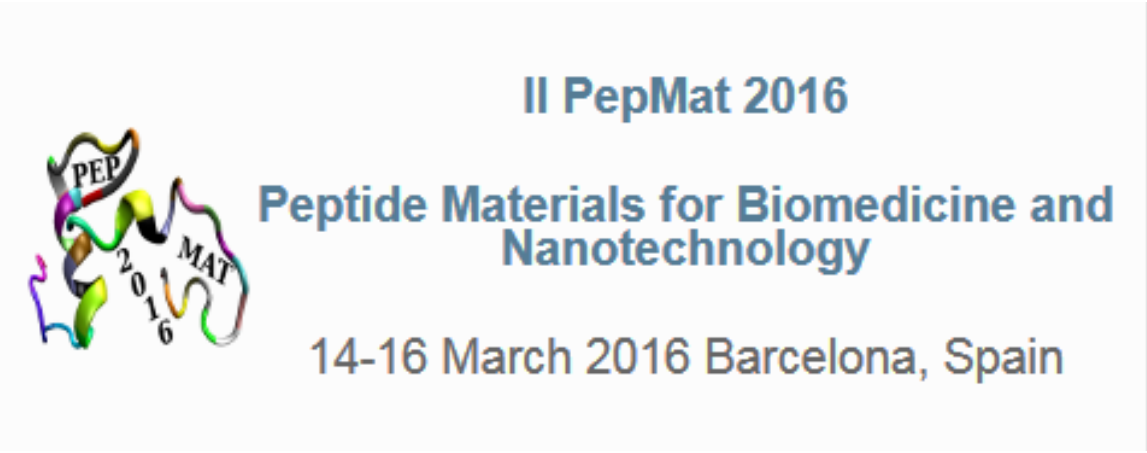
Ultrashort Self-assembled Peptides for Biomaterial Applications

Dr Garry Laverty

School of Pharmacy

Biofunctional Nanomaterials Group

Queen's University Belfast

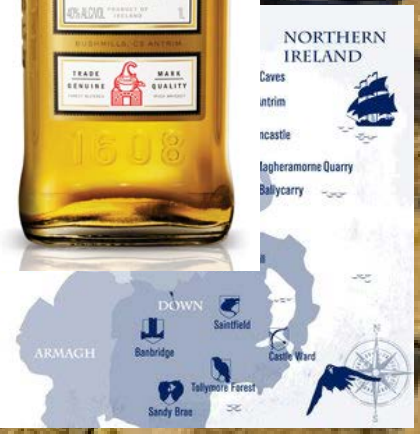


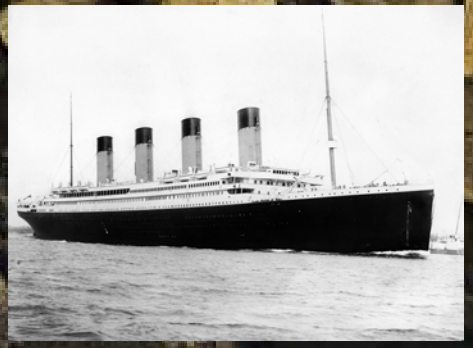
Queen's University Belfast

SCHOOL OF
Pharmacy

No. 1 Again!!

The School of Pharmacy is ranked as the No.1 Pharmacy School in the UK by the 2016 Times and Sunday Times Good University Guide (for the second successive year)





Current Peptide Interests

Chem Biol Drug Des 2010; **75:** 563–569
Research Article

doi: 10.1111/j.1747-0285.2010.00973.x

Antimicrobial Activity of Short, Synthetic Cationic Lipopeptides

Garry Laverty, Martin McLaughlin, Christopher Shaw, Sean P. Gorman and Brendan F. Gilmore*

Biomaterials Research Group, School of Pharmacy, Queens University of Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast, BT9 7BL, UK
**Corresponding author: Dr Brendan F. Gilmore, b.gilmore@qub.ac.uk*

for new antimicrobial agents with activity against pathogens that are resistant to the available armoury of antibiotics (3,4).

One class of compounds that has attracted increasing attention in the last two decades are the cationic antimicrobial peptides (AMPs). Antimicrobial peptides are short (typically ranging from 12 to 100 amino acid residues in length), generally exhibit rapid and efficient antimicrobial toxicity against a range of pathogens (5,6) and constitute critical effector molecules in the innate immune sys-

Society For Biomaterials

Antimicrobial peptide incorporated poly(2-hydroxyethyl methacrylate) hydrogels for the prevention of *Staphylococcus epidermidis*-associated biomaterial infections

Garry Laverty, Sean P. Gorman, Brendan F. Gilmore
Biomaterials Research Group, School of Pharmacy, Queens University of Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, United Kingdom

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Published online 4 April 2012 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jbm.a.34132

Int. J. Mol. Sci. **2011**, *12*, 6566-6596; doi:10.3390/ijms12106566

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ISSN 1422-0067
www.mdpi.com/journal/ijms

Review

The Potential of Antimicrobial Peptides as Biocides

Garry Laverty [1,*], Sean P. Gorman [2] and Brendan F. Gilmore [2]

Chem Biol Drug Des 2015; **85:** 645–652
Research Letter

Biofilm Eradication Kinetics of the Ultrashort Lipopeptide C_{12}-OOWW-NH_2 Utilizing a Modified MBEC Assay™

Garry Laverty*, Sean P. Gorman and Brendan F. Gilmore

Biomaterials, Biofilm and Infection Control Research Group, School of Pharmacy, Queens University of Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, UK
**Corresponding author: Garry Laverty, garry.laverty@qub.ac.uk*

Cationic antimicrobial peptides exist throughout the nature as defense mechanisms in both prokaryotic and eukaryotic organisms. Antimicrobial peptides have evolved over millennia to become inherent antimicrobial molecules and effective mediators of the innate and adaptive immune response (1). They have been proven to be effective at neutralizing Gram-negative bacterial lipopolysaccharide endotoxin and aid the process of wound healing (2,3). Varying in the length of their primary sequence, frequently

Research article
Journal of PeptideScience
Received: 13 May 2015 Revised: 26 June 2015 Accepted: 6 July 2015 Published online in Wiley Online Library
(wileyonlinelibrary.com) DOI 10.1002/psc.2805

Anti-biofilm activity of ultrashort cinnamic acid peptide derivatives against medical device-related pathogens

Garry Laverty,* Alice P. McCloskey, Sean P. Gorman and Brendan F. Gilmore

Research Article
SOJ Microbiology & Infectious Diseases

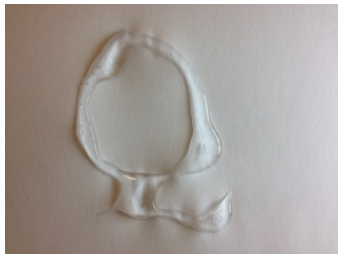
Cationic Antimicrobial Peptide Cytotoxicity

Garry Laverty* and Brendan Gilmore

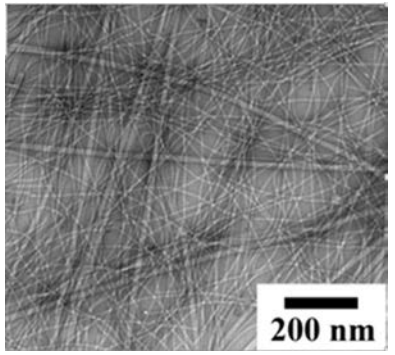
Rational Design of Antimicrobial Peptide Motif vs Self-assembly

Antimicrobial Activity	Propensity to Self-assembled hydrogels
Hydrophobic/Hydrophilic (Charge) ratio (more important with regard to antimicrobial activity than size)	**Hydrophobic/Hydrophilic balance**
Interactions with microbial extracelluar membranes	Non Covalent intermolecular interactions (e.g. Van der Waal's, π-π stacking)
Interaction with intracellular targets/processes (DNA, RNA, enzymes, protein synthesis)	Ability of peptide to form hydrogen bonds with each other and with water

McCloskey A.P., Gilmore, B.F., Laverty, G. (2014) Evolution of Antimicrobial Peptides to Self-Assembled Peptides for Biomaterial Applications. *Pathogens*. *3*(4); 791-821.

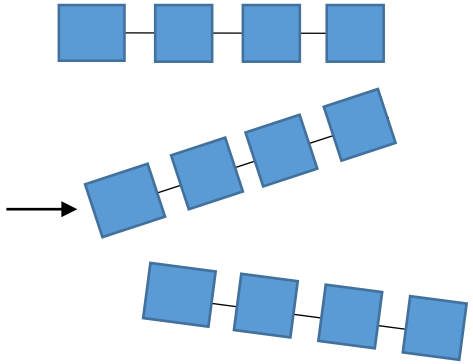


Core Technology



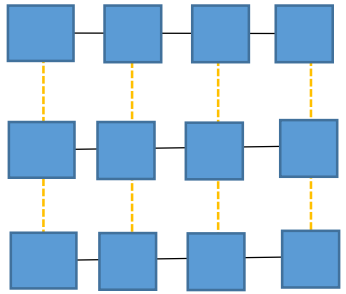
Peptide Hydrogels

Self-assembled Peptides



Stimuli

-pH
-Temperature
-Ionic Strength
-Specific enzymes



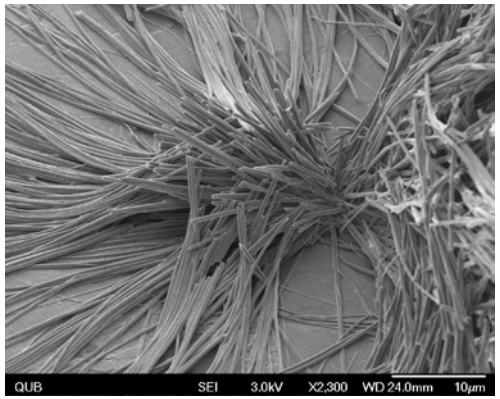
Self-assembly

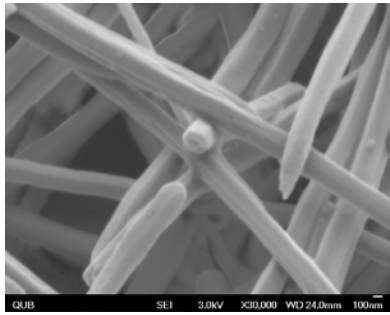
Peptide

Nanotubes

Short peptide sequences

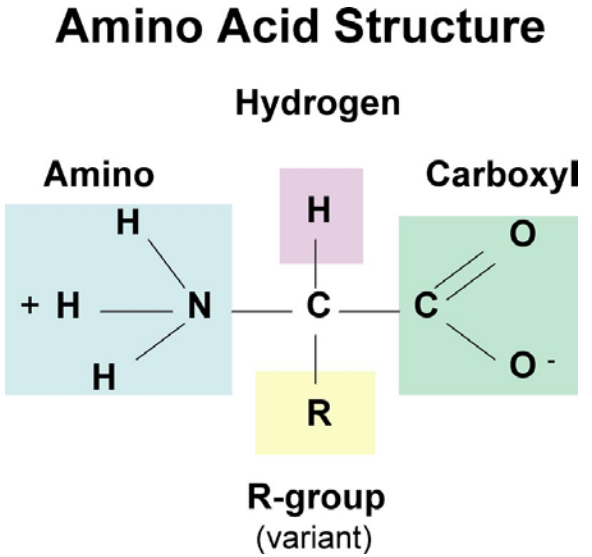
Non assembled





Advantages of Ultrashort Peptides

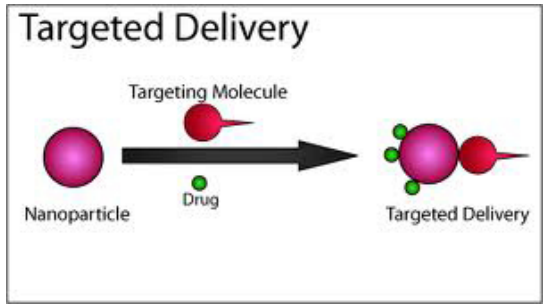
- Successful in producing a series peptide sequences of that self-assemble to form hydrogels or nanotubes in response to physiological stimuli
- Ultrashort peptides (< 7 amino acids) → More cost effective → Upscale by Pharmaceutical Industry → Increased translational potential → Patient benefit
- Numerous advantages over current synthetic materials including:
  - Increased chemical versatility
  - Minimal immunogenicity and enhanced biocompatibility
  - Tunable biodegradability
  - Tailored self-assembly/pharmacological properties (e.g. antimicrobial) in response to stimuli



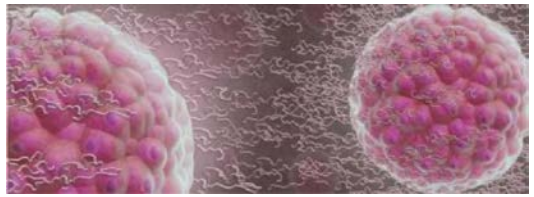
Biofunctional Nanomaterials Utilising the Building Blocks of Life!

Infection and Medical Devices

Wound healing



Drug Delivery



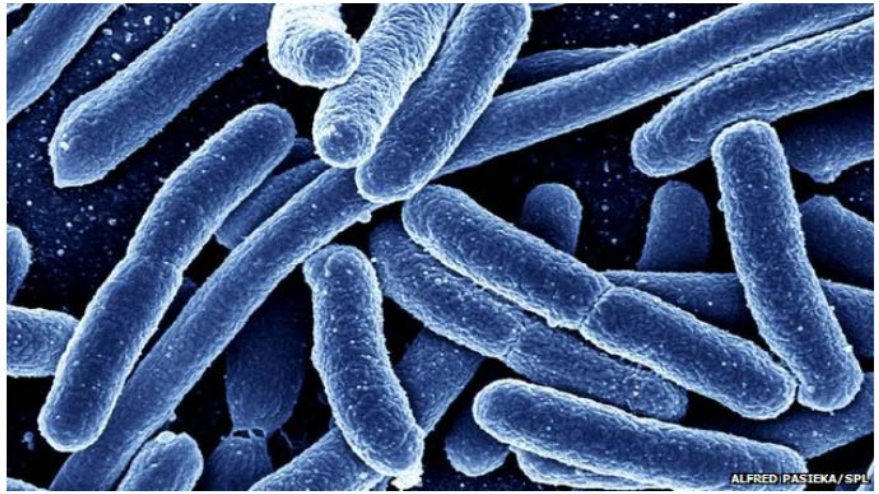
Stem Cells/Regenerative medicine

Antimicrobial Resistance

BBC NEWS

Superbugs to kill 'more than cancer' by 2050

COMMENTS (565)



Drug resistant E.coli bacteria are already a significant problem in Europe

Drug resistant infections will kill an extra 10 million people a year worldwide - more than currently die from cancer - by 2050 unless action is taken, a study says.

They are currently implicated in 700,000 deaths each year.

Related Stories

Analysis: Antibiotic apocalypse

- Medical device related infections
- Increased reservoir of “superbugs”
- Persistent burden on:
  - Patient morbidity & mortality
  - Family and carers
  - Healthcare budgets

sky NEWS

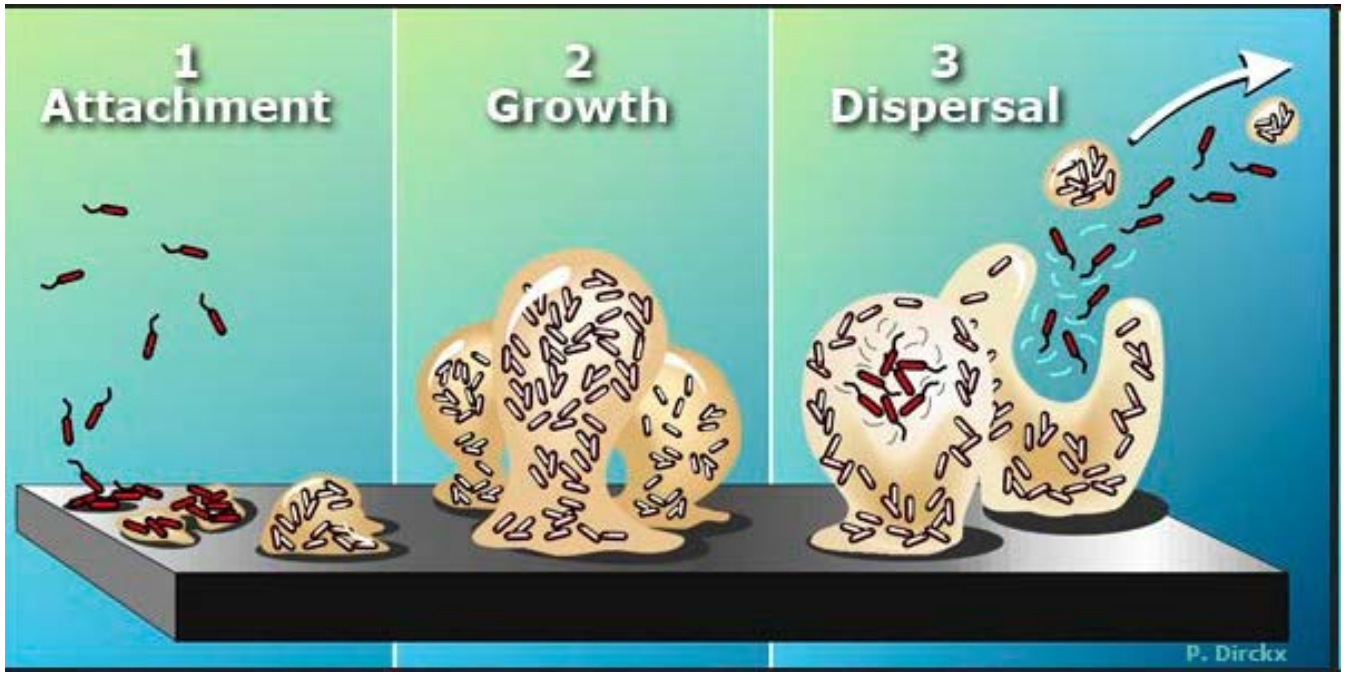
Superbugs 'Could Send UK Back To The Dark Ages'

Action is needed to stop the world entering a post-antibiotic era in which common infections and injuries can kill, say experts.

17:49, UK,
Wednesday 02 July 2014

Planktonic vs. Biofilm Bacteria

- Planktonic form: Free floating in liquid
- Biofilm form: sessile, composed of aggregated microcolonies of cells surrounded by a protective extracellular polymeric matrix
- Mature biofilms can resist 10-1000 times the concentrations of standard antibiotic regimens that are required to kill genetically equivalent planktonic forms



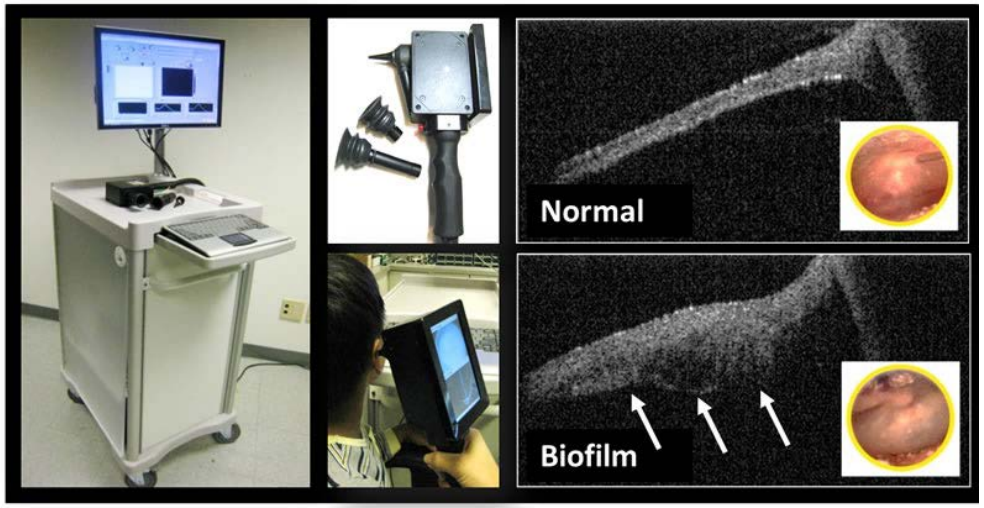
P. Dirckx, Centre for Biofilm Engineering, Montana State University, Bozeman

Biofilms and Implant-Associated Infections. Laverty, G., Gorman, S.P. and Gilmore, B.F. In: Biomaterials and Medical Device Associated Infections. Woodhead Publishing Ltd. 2014.

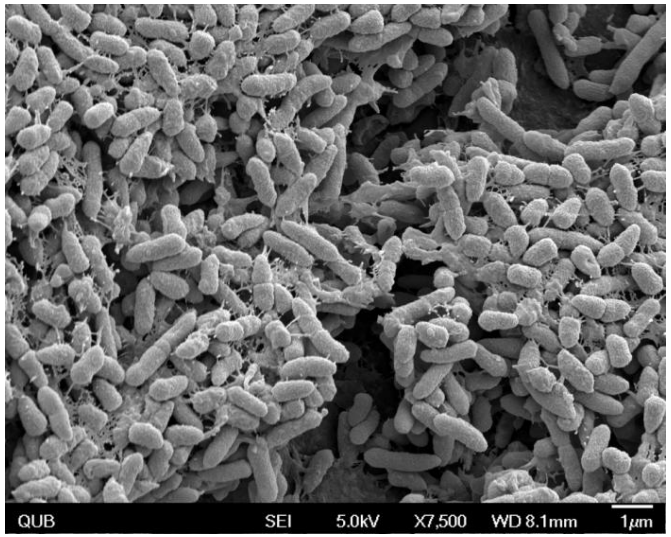
Biofilms in the Environment and Medicine

Biofilm growth on rocks in a stream (USGS) and within a kitchen pipe (MSU Center for Biofilm Engineering).

Biofilm formation on a voice prosthesis implant.



University of Illinois researchers tested a prototype of a new device that can see biofilms behind the eardrum to better diagnose and treat chronic ear infections.

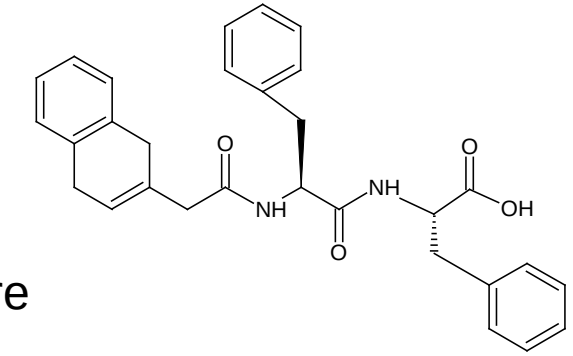


SEM Pseudomonas aeruginosa, shown here attached to an implant surface, is one of many resistant microorganisms

Self-assembled Ultrashort Peptide Gels

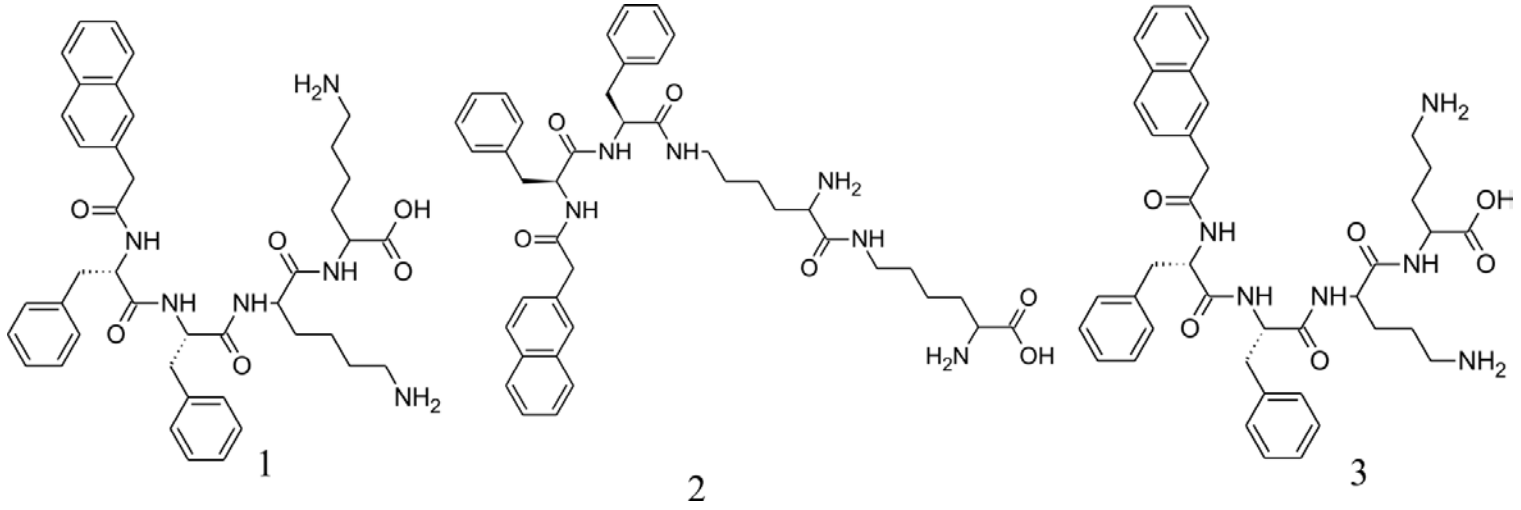
- 2013 Research Placement Prof. Bing Xu Lab, School of Chemistry, Brandeis, Waltham, Boston
- Successful in producing a series of ultrashort peptides (< 7 amino acids) that self-assembled at physiological pH
- (X_1-FF-X_2)
- More cost effective
- Hydrophobicity provided by inclusion of a naphthalene (Nap) grouping (at X_1 position) and varying quantity of phenylalanine in primary structure

NapFF structure



Ultrashort Cationic Variants: Primary Structures

- Charge: Inclusion of cationic amino acids
  - 1) Lysine
  - 2) Ornithine
  - 3) epsilon (ε) Lysine
- Minimum of 2 charged units required for antimicrobial activity
- Primary amine group provides cationic charge
- Cationic amino acids vary by number of methylene units on R-group

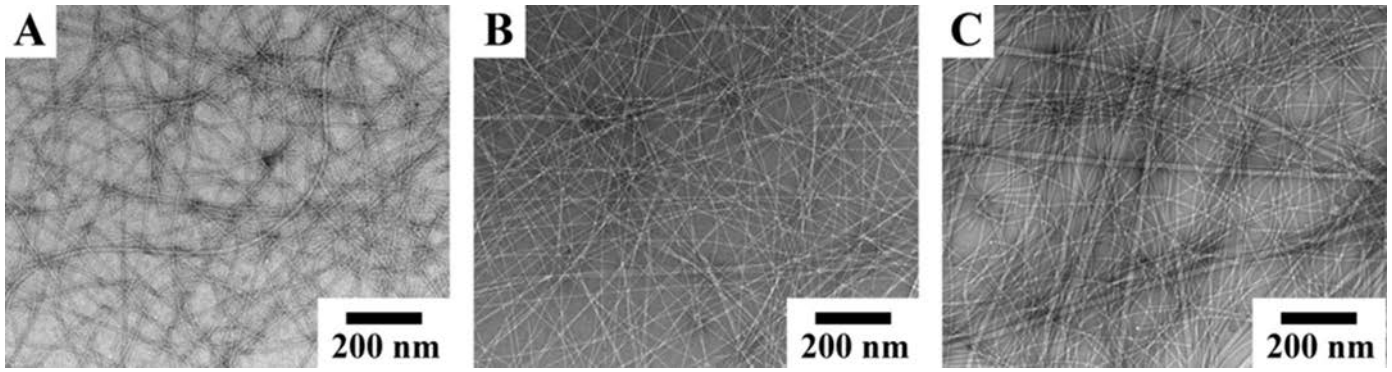


-Laverty, G., McCloskey A.P., Gilmore, B.F., Jones, D.S., Zhou, J., Xu, B (2014). Ultrashort Cationic Naphthalene derived Self-assembled Peptides as Antimicrobial Nanomaterials. *Biomacromolecules*; 15: 3429−3439.
-Laverty, G., Gorman, S.P. and Gilmore, B.F (2012). The Adherence of *Staphylococcus epidermidis* to Antimicrobial Peptide Incorporated poly(2-hydroxyethyl methacrylate) Hydrogels. *Journal of Biomedical Materials Research: Part A* 100A; 1803–1814.

Ultrashort Cationic Variants: Self-assembly

- Form Self supporting hydrogels at pH 7.4: pH triggering method

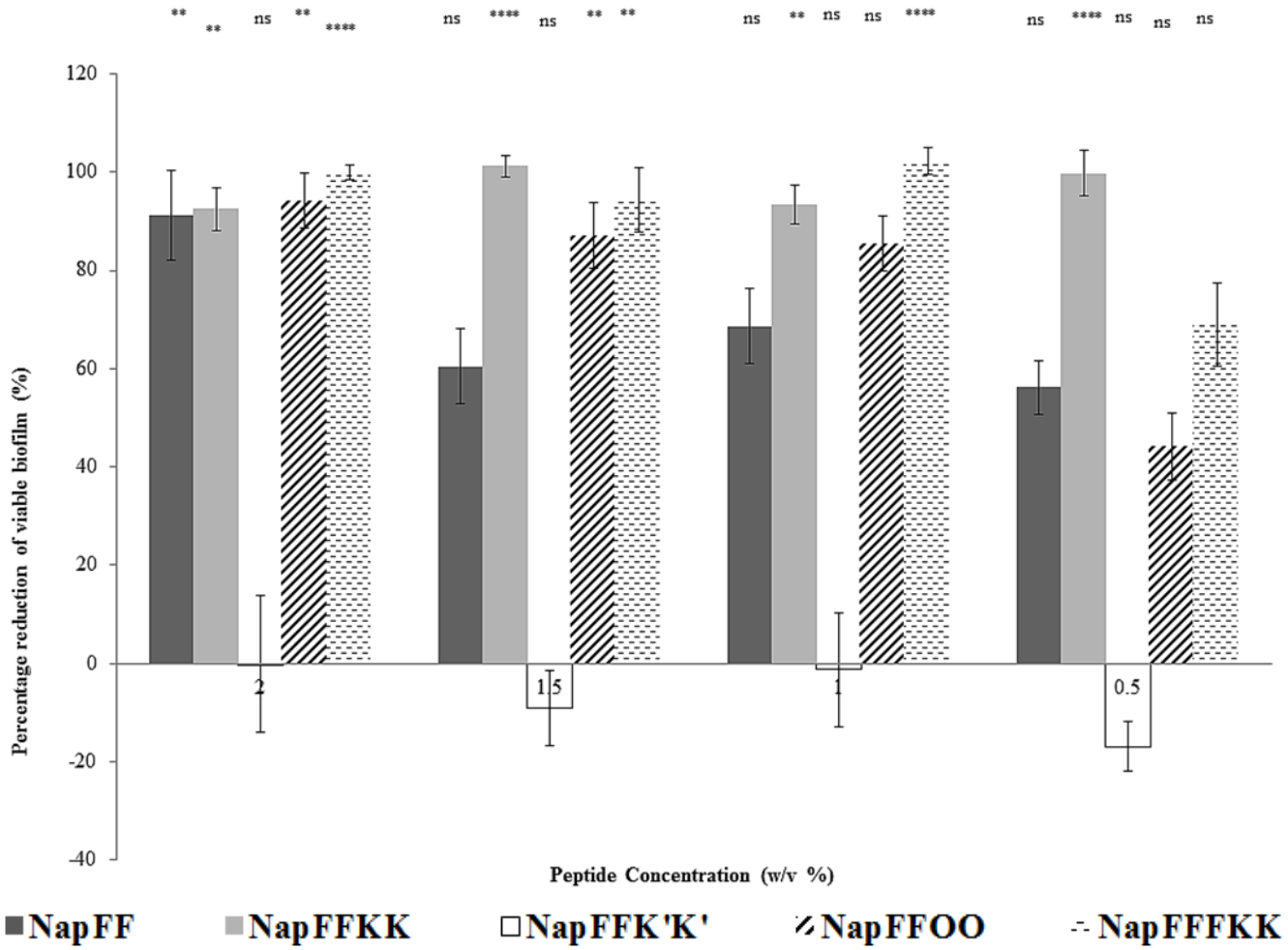
Optical images of gel (A) NapFFOO, (B) NapFFKK, (C) NapFFεKεK, at a concentration of 1% w/v and pH of 7.4 in water



Transmission electron microscopy (TEM) images of (A) NapFFOO, (B) NapFFKK, (C) NapFFεKεK, at a concentration of 1% w/v and pH of 7.4 in water

Laverty, G., McCloskey A.P., Gilmore, B.F., Jones, D.S., Zhou, J., Xu, B (2014). Ultrashort Cationic Naphthalene derived Self-assembled Peptides as Antimicrobial Nanomaterials. *Biomacromolecules*; 15: 3429−3439.

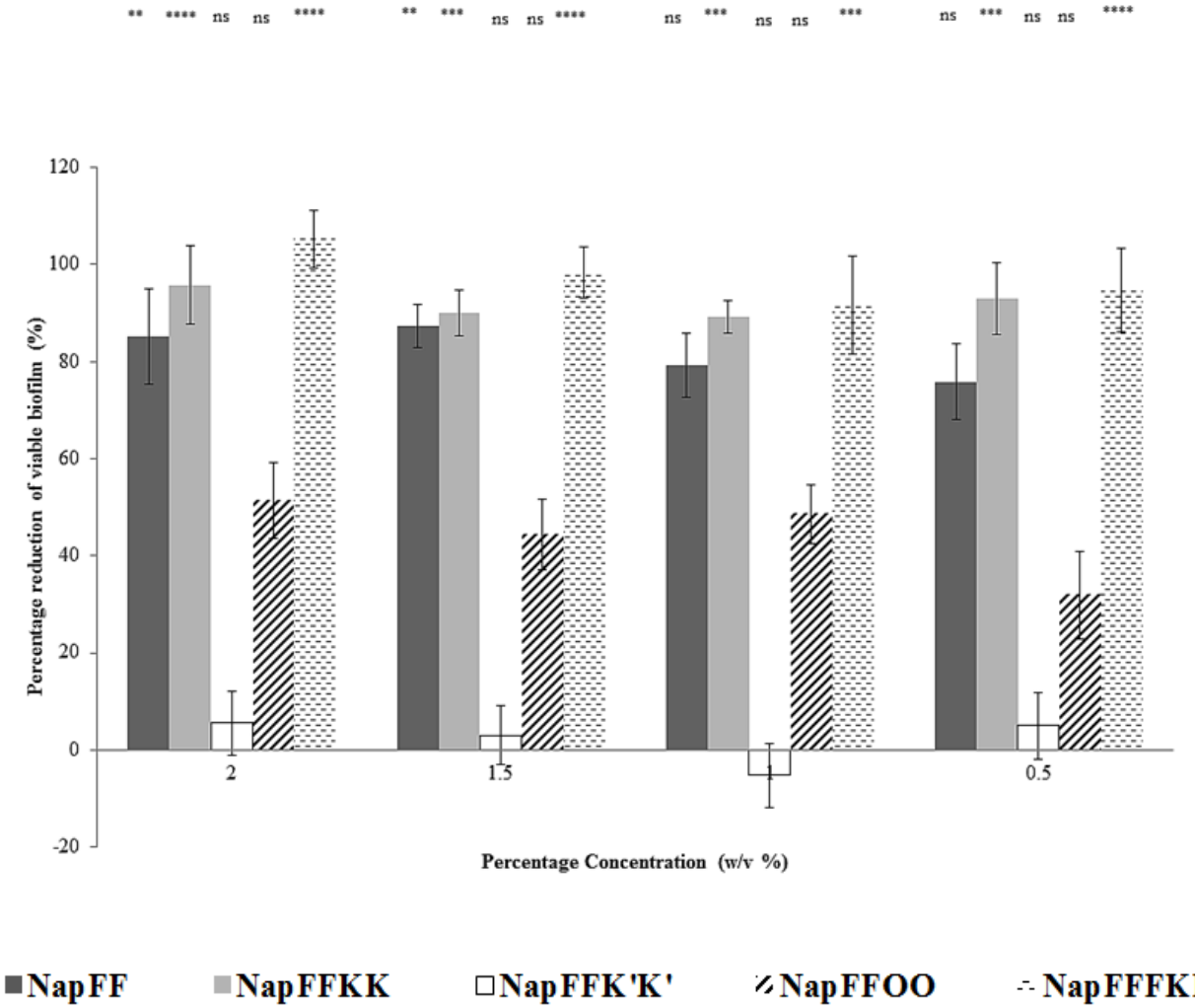
Anti-Biofilm Activity Gram-positive Bacteria



Percentage reduction of mature 24 hour *Staphylococcus aureus* (ATCC 29213) biofilm after 24 hour incubation with naphthalene derived self-assembled hydrogels utilizing an alamarBlue assay. Results are displayed as a mean of 8 replicates

Laverty, G., McCloskey A.P., Gilmore, B.F., Jones, D.S., Zhou, J., Xu, B (2014). *Biomacromolecules*; **15**: 3429−3439.

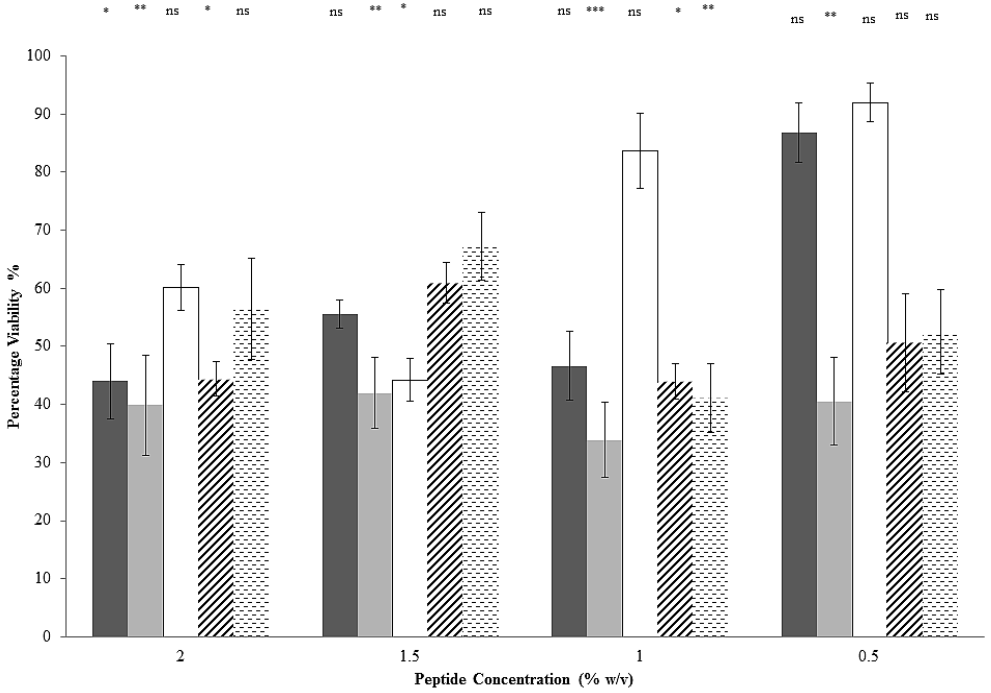
Anti-Biofilm Activity Gram-negative Bacteria



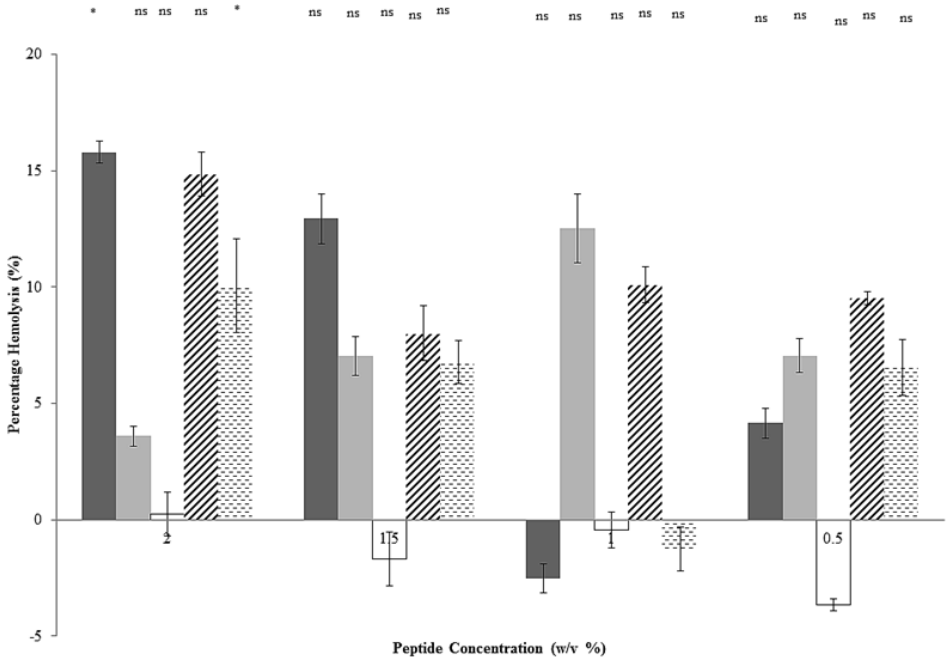
Percentage reduction of mature 24 hour *Escherichia coli* (NCTC 11303) biofilm after 24 hour incubation with naphthalene derived self-assembled hydrogels utilizing an alamarBlue assay. Results are displayed as a mean of 8 replicates

Laverty, G., McCloskey A.P., Gilmore, B.F., Jones, D.S., Zhou, J., Xu, B (2014). *Biomacromolecules*; 15: 3429−3439.

Toxicity: Tissue Culture & Haemolysis



Percentage viability of CCL 1 [NCTC clone 929]-murine fibroblasts subcutaneous connective tissue monolayer cells after 24 hour exposure to naphthalene derived self-assembled hydrogels utilizing an alamarBlue assay. Results are displayed as a mean of 8 replicates



Percentage hemolysis of the naphthalene peptides against equine erythrocytes. Each value is expressed as the mean of six replicates, incubated at 37 °C for 1 hour

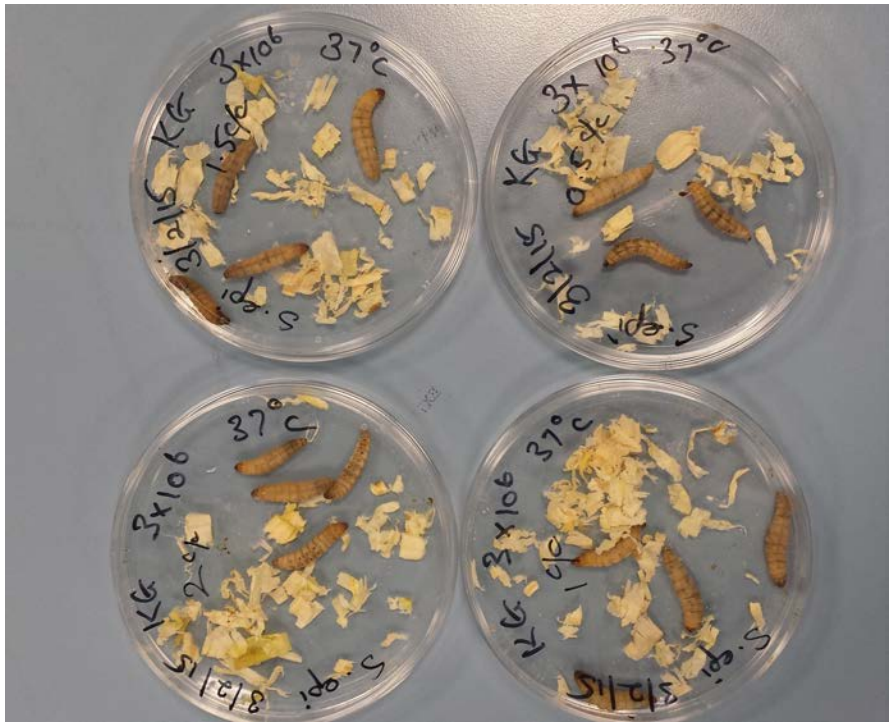
NapFF NapFFKK NapFFK'K' NapFFOO NapFFFKK

Laverty, G., McCloskey A.P., Gilmore, B.F., Jones, D.S., Zhou, J., Xu, B (2014). *Biomacromolecules*; 15: 3429−3439.

Galleria mellonella (Waxworm) assay

National Centre for the Replacement Refinement & Reduction of Animals in Research

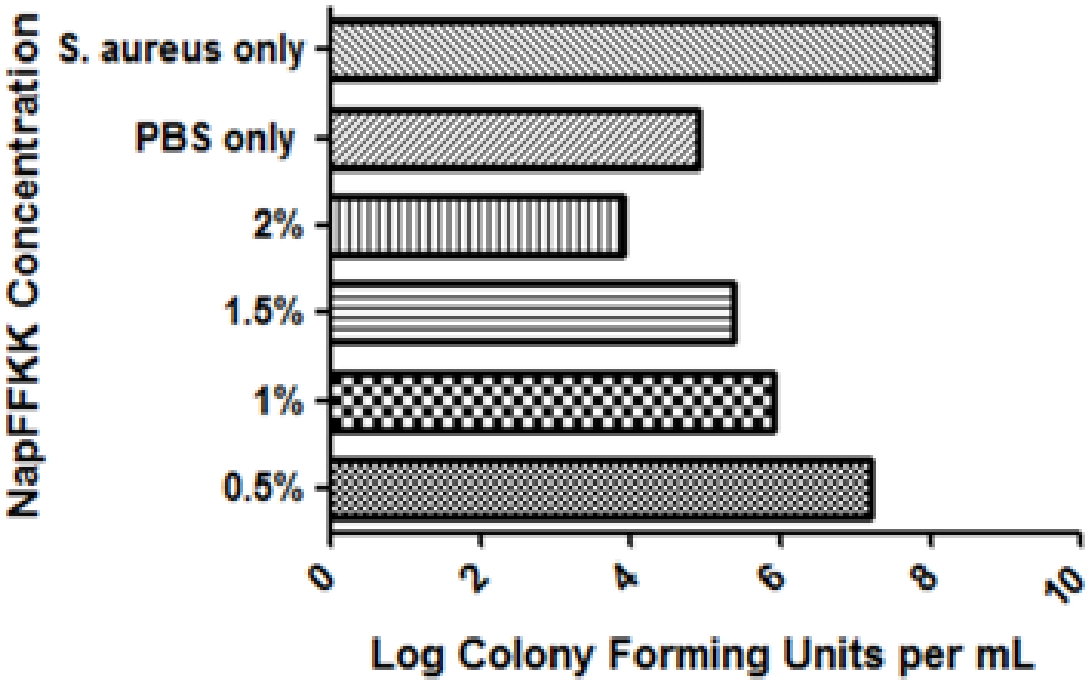
Adult *Galleria mellonella* being inoculated via the pro-leg.



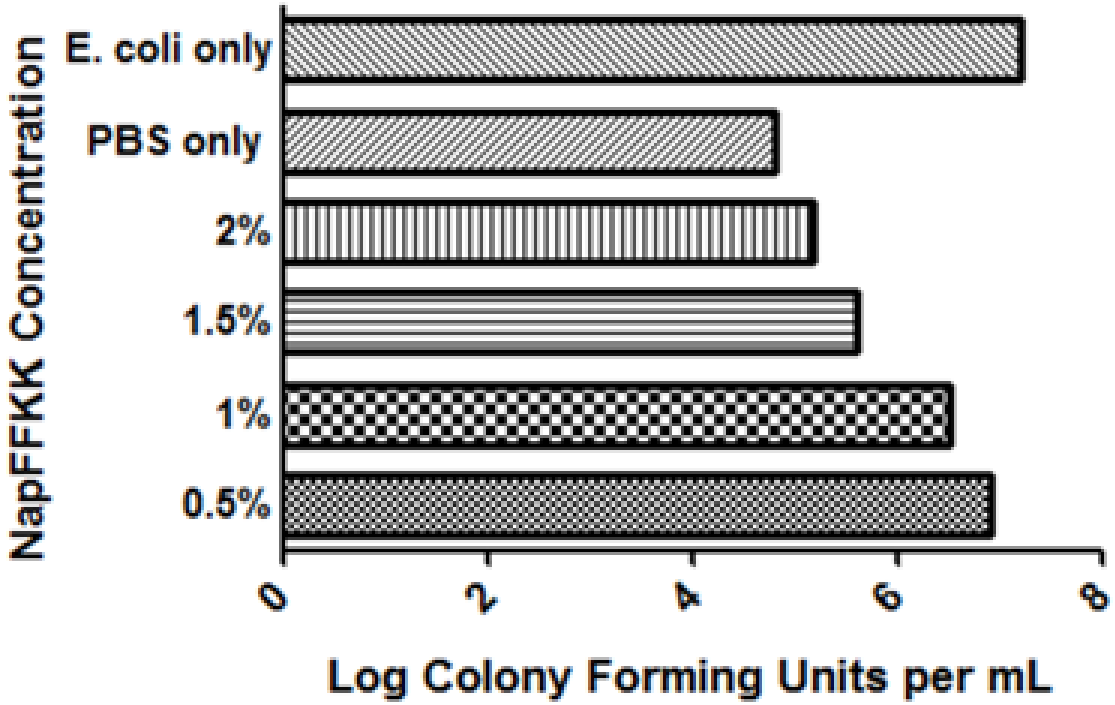
Non viable *Galleria mellonella*

Data demonstrates biocompatibility (NapFFKK) and reduction in bacterial load with *S.aureus* (ATCC 29213) *S.epidermidis* (ATCC 35984), *E. coli* (NCTC 11303) and *Pseudomonas aeruginosa* (PAO1)

S.aureus



E.coli



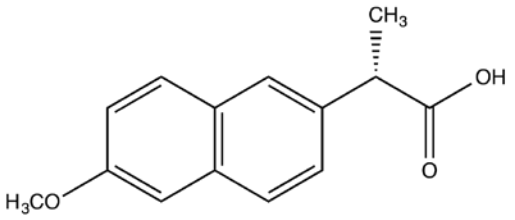
Bacterial counts (Log CFU/mL) from haemolymph extracted 72 hour following inoculation with 20µL of 1x 10^5 bacteria and treatment 2 hours later with 20µL NapFFKK.

McCloskey, A.P., Lee, M., Gilmore, B.F., Laverty, G*. *Galleria mellonella* as an *in vivo* infection model: the treatment of medical device related pathogens by Ultrashort Self-assembled Peptides. *Nanomedicine. In preparation*

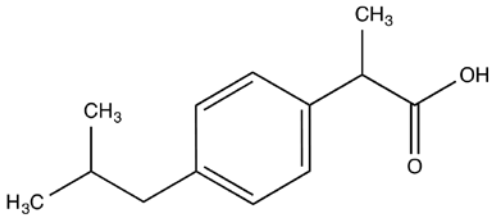
Dual Antimicrobial Anti-inflammatory Nanomaterials

Poster 16

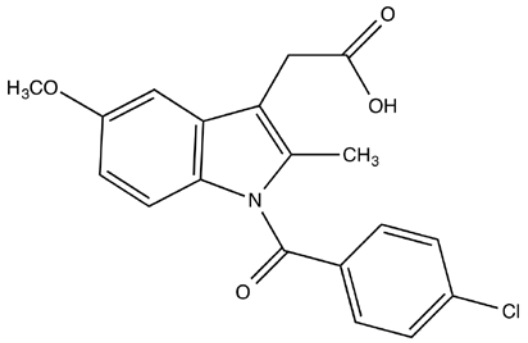
- Hydrophobicity provided by NSAID structure
- High in aromaticity
- Display self-assembly and gelation characteristics
- Potential applications in chronic infected wounds



Naproxen



Ibuprofen

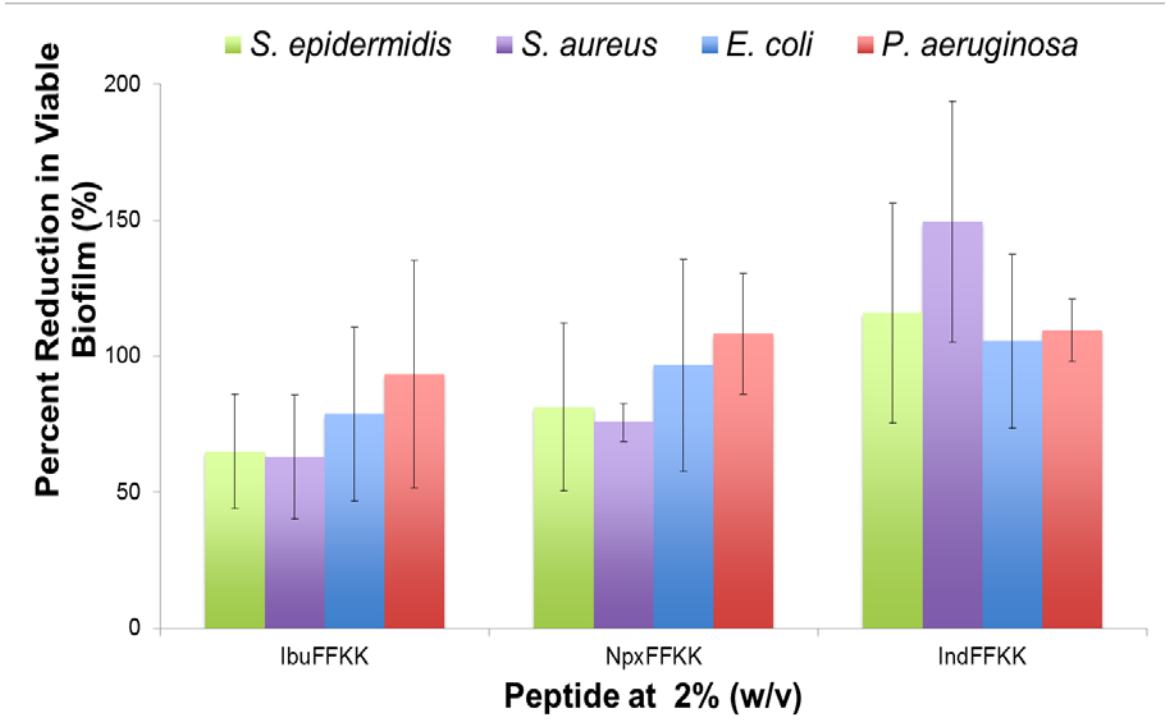


Indometacin

A.P. McCloskey, S.M. Gilmore, J. Zhou, L. Chen, E.R. Draper, B.F. Gilmore, D. Adams, B. Xu, G. Laverty*. Self-assembling Ultrashort NSAID Peptidomimetics: Bifunctional Antimicrobial and Anti-inflammatory Nanomaterials. *Adv.Healthc.Mater*. *In preparation.*

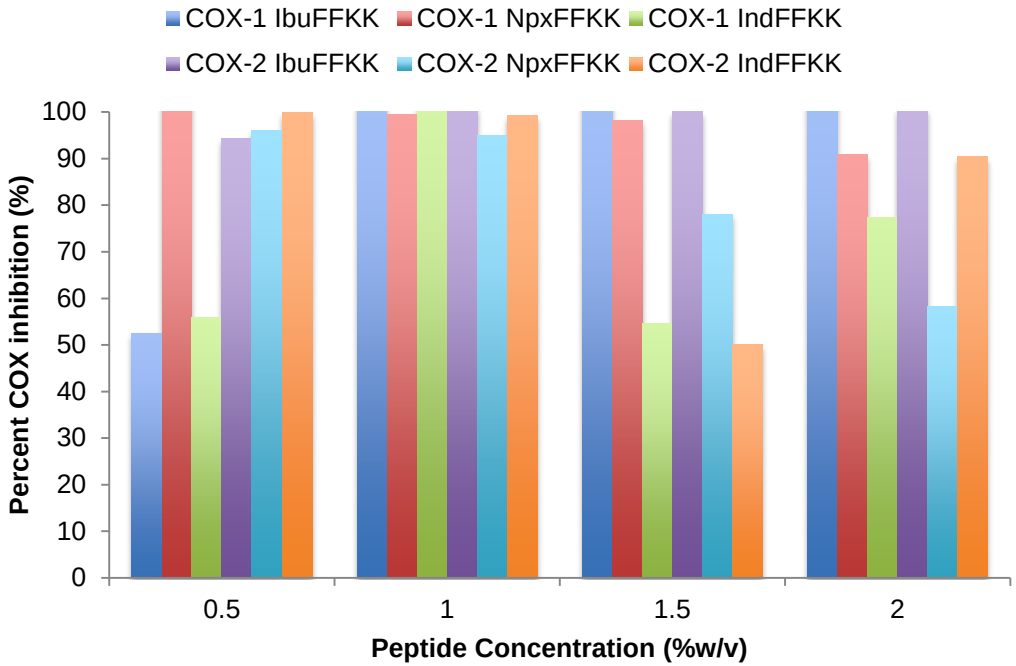
Dual action

Antimicrobial



Percentage reduction of mature 24h biofilm treated with 2% w/v NSAID- conjugated hydrogels utilizing an alamarBlue assay.

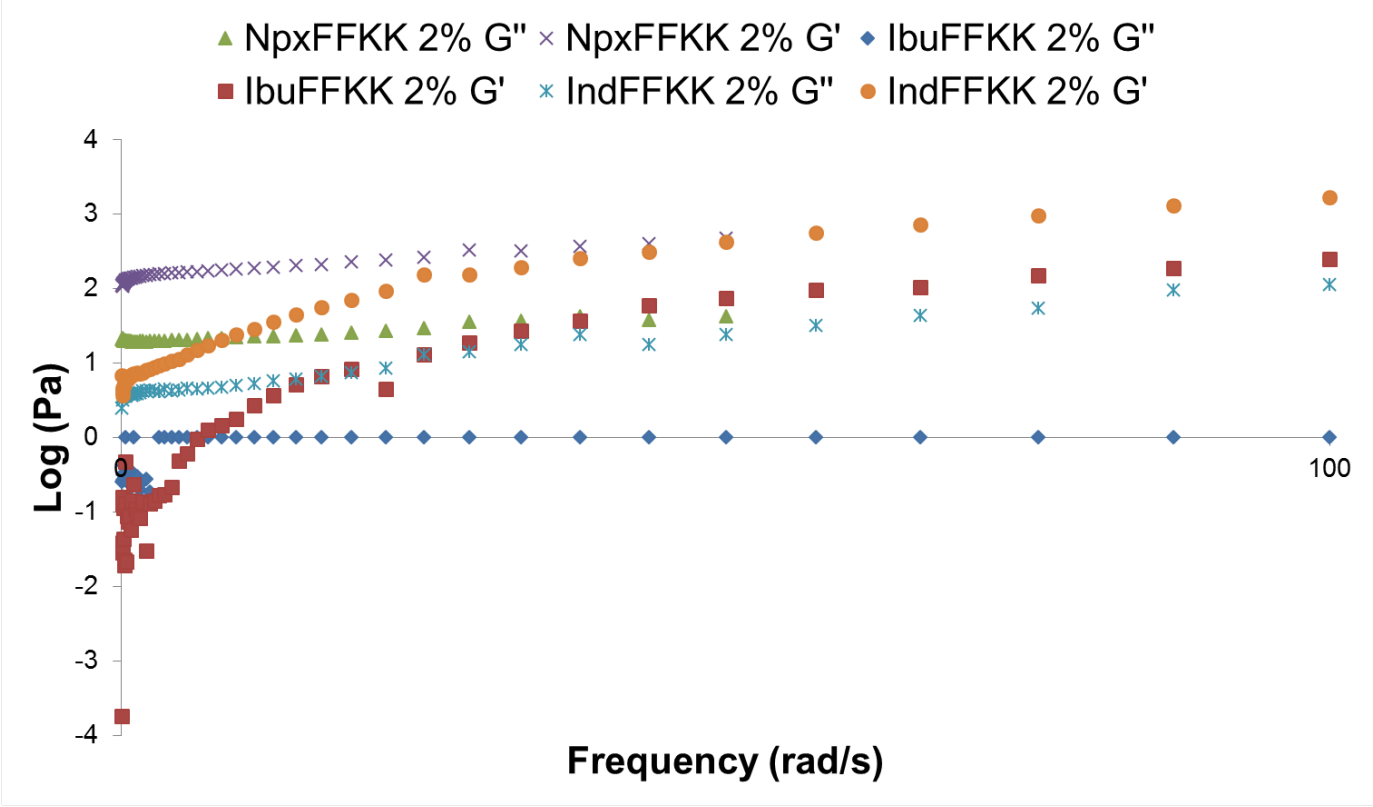
Anti-inflammatory



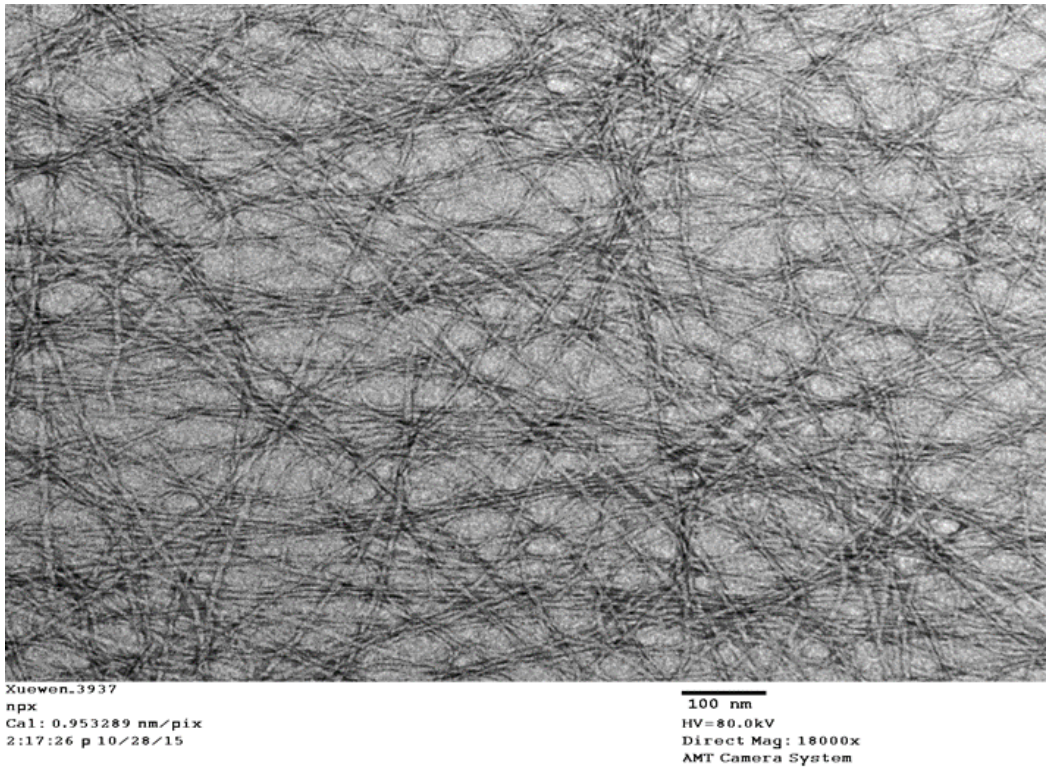
Percent inhibition of COX 1 and 2 enzyme by NSAID self-assembled hydrogels and by the model COX inhibitor DuP-697 using a COX Fluorescent Inhibitor Screening Assay Kit.

Queen's University Belfast

Dual Antimicrobial Anti-inflammatory Nanomaterials



Oscillatory rheology



TEM-Naproxen-FFKK-OH

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Potential Applications: Antimicrobial Platforms

- Prevention of Biomaterial/Medical Device/Implant Infections:
  - The medical device industry has an estimated worth of US$75 billion worldwide
  - Urinary catheters
  - Intravascular catheters
  - Hip replacements
  - Cardiac devices

  Implant surface provides perfect environment for growth of resistant pathogens!

Urinary catheter encrustation

- Wound healing/surgical gel: Increased healing as mimics natural tissues

- Platforms/vehicles to deliver existing antimicrobials, extend spectrum of activity to Gram-negatives
- Translation for patient benefit

Queen's University Belfast

Thank You!

- Alice McCloskey (DEL funded PhD student): Biomaterials
- Rawan Huwaitat (PhD student): Gram-negative nanotubes
- Simon Porter (DEL funded PhD student) Nanotubes cancer
- Alyaa Albadr (PhD student) Ocular nano
- Giovanni Cristini (Erasmus student)
- Dr Hema Nagaraj (Visiting Research Fellow)
- Lucia Murias (Visiting Research Assistant)
- Sophie Gilmore (S*f*am: Students into Work)
- Merissa Lee (S*f*am: Students into Work)

http://lavertylab.weebly.com

- The Xu Group, School of Chemistry, Brandeis.
- The Adams Lab, Department of Chemistry, University of Liverpool

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